Serum Vitellogenin Levels and Reproductive Impairment of Male Japanese Medaka (*Oryzias latipes*) Exposed to 4-*tert*-Octylphenol

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The induction of synthesis of the "female" yolk precursor protein vitellogenin (VTG) in male fish by estrogenic chemicals in the environment has been demonstrated in many recent reports. However, little is known about the organismal and biological significance of this phenomenon. To examine the relationship between VTG production in male fish and reproductive impairment, adult male medaka were exposed to 4-*tert*-octylphenol (OP), a known environmental estrogen, in concentrations ranging from 20 to 230 ppb for 21 days, under flow-through conditions. Following exposure, male fish were mated, in the absence of OP, with unexposed females. Breeding groups composed of exposed males and control females produced about 50% fewer eggs than control groups. VTG levels in serum of male fish increased with increasing OP exposure concentration and decreased after OP exposure was discontinued. Nevertheless, significant correlations (p<0.01) were observed between VTG levels in exposed male fish and 1) OP exposure concentrations, 2) percent of fertilized eggs, and 3) survival of embryos. OP-induced VTG synthesis and reproductive impairment appear to be closely linked phenomena. Histological examination indicated spermatogenesis in OP-exposed fish was inhibited, and some exposed fish had oocytes in their testes. Finally, OP caused a significant increase in the number of abnormally developing embryos, suggesting that OP may be genotoxic as well as estrogenic. *Key words*: medaka, octylphenol, reproduction, vitellogenin, xenoestrogen. *Environ Health Perspect* 107:385–390 (1999). [Online 2 April 1999]
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A growing body of scientific evidence suggests that a wide range of chemicals introduced into the (aquatic) environment by humans may be producing adverse health effects in humans and wildlife species by disrupting endocrine system function. Chemicals considered to interfere with hormone function include environmentally persistent organochlorines [polychlorinated biphenyls (PCBs), DDT, dioxins, furans, pentachlorophenol, hexachlorobenzene], polycyclic aromatic hydrocarbons (PAHs), herbicides (alachlor, atrazine), fungicides (tributyl tin, vinclozolin), insecticides and nematocides (aldicarb, chlordane, dieldrin, endosulfan, lindane, toxaphene, pyrethroids), pharmaceuticals [drug estrogens, birth control pills, diethylstilbestrol (DES)], nonionic surfactants (alkylphenol polyethoxylates, *p*-octylphenol and *p*-nonylphenol), products associated with plastics (bisphenol A, phtalates), and heavy metals such as cadmium, lead, and mercury (*1–4*).

Environmental estrogens, or xenoestrogens, chemicals with bioactivity similar to the endogenous female hormone estrogen, are known to affect development and sexual maturation of (in)vertebrates. Xenoestrogens can exert their action by binding to the cell's estrogen receptor (ER), but they can also act through ER-independent mechanisms (*5*). Reported adverse effects in humans include increased incidences of breast cancer and reduced sperm counts, whereas wildlife populations affected by xenoestrogens display a variety of reproductive alterations such as cryptorchidism in the Florida panther, small baculum in young male otters, small penises in alligators, sex reversal in fish, and egg-shell thinning and altered social behavior in birds (*3,4,6–8*).

Alkylphenol polyethoxylates (APEs) are nonionic surfactants widely used in the manufacturing of cleaning agents, plastics, paper, cosmetics, and food products (*9*). APEs are discharged from industrial wastewater as nontoxic, hydrophilic compounds. However, bacteria metabolize APEs into hydrophobic, estrogenic by-products, including *p*-nonylphenol and 4-*tert*-octylphenol (OP), that bioaccumulate in aquatic wildlife and may affect reproductive ability (*10,11*). These metabolites bind to the ER of fish and mammals (*12–14*), induce transcriptional activation of estrogen-responsive genes (*15*), and induce production of the yolk protein vitellogenin (VTG) in fish hepatocyte cell culture and in male rainbow trout (*16–20*). Of the alkylphenols examined, OP appears to be the most biologically active (*9*).

VTG is normally synthesized in the liver of adult female egg-laying vertebrates (*21*). Therefore, when detected in the serum of male fish, VTG can be used as a biomarker of exposure to estrogenic chemicals (*22,23*). Several researchers have demonstrated that exposure of male fish, turtles, and frogs to (xeno)estrogenic chemicals results in the induction of VTG synthesis (*17,24–28*), but evidence linking VTG levels in serum of male animals to reproductive impairment is scarce. The purpose of this study was to determine if the presence of VTG in the serum of male Japanese Medaka (*Oryzias latipes*) exposed to OP can be correlated with decreased reproductive success and survival of the F1 generation.

Materials and Methods

Test organism. The Japanese medaka (*Oryzias latipes*) used in this study were obtained from broodstock cultured and maintained for over 10 years at our laboratory. Male medaka selected for exposure to OP were approximately 6 months post hatch and fully mature.

Test chemical. The test substance, 4-*tert*-octylphenol (>97% pure), was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wisconsin. The study stock solution was prepared by dissolving 1.5 g OP in 3 ml methanol, then diluted to 1 liter with triethylene glycol.

Exposure conditions. Concentrations of OP for the exposures were selected from a preliminary 21-day OP exposure/reproductive study and a 96-hr acute toxicity test. In these earlier exposures, concentrations of 5 ppb OP had no effect on reproduction or on embryo survival, and toxicity was not observed below 790 ppb. Based on these data, concentrations of 20, 50, 100, and 300 ppb OP were selected for the study presented here. Dilution water for exposure and culture was from a 177-m nonchlorinated well located on site. The water was particle and carbon filtered, temperature adjusted, and aerated prior to introduction into the test aquaria. Duplicate 20-liter aquaria per treatment and

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control, housing 25 male medaka each, received the aforementioned OP concentrations for a period of 21 days in an intermittent flow-through chamber similar to that described by Walker et al. (*29*). The flow rate was maintained at 100 l/aquarium/day. The exposure system provided a 16-hr light:8-hr dark photoperiod within an isolation chamber used to protect fish from unnecessary disturbance while housed in a heated recirculating water bath to maintain constant temperature. Fish were fed AquaTox Special dry flakes (Sigler Bros, Gardner, PA) and brine shrimp nauplii once daily. Temperature, dissolved oxygen (DO), and pH of each treatment and control aquarium were measured twice weekly. Temperature was maintained at 27 ± 1°C. DO was 5.3 ± 0.89 ppm, and pH was 8.7 ± 0.1.

Octylphenol analysis. The OP concentration in each aquarium was measured twice weekly during the 21-day exposure. One-liter samples were taken from each aquarium, amended with internal standard (*n*-octylphenol), and adjusted to pH 2 with HCl. Samples were vacuum filtered through preconditioned Varian Bond-Elut PPL solid phase extraction cartridges (Varian Sample Preparation Products, Harbor City, CA). The OP and internal standard were eluted from the cartridges with ethyl acetate and injected into a Perkin Elmer GC/FID system (Perkin-Elmer, Norwalk, CT). OP concentrations were calculated from linear standard calibrations curves.

Vitellogenin analysis. Following exposure, 10 fish from each treatment and control group (five per replicate) were anesthetized with tricaine methanesulfonate (MS-222) and bled by cutting a gill arc. Blood was collected by capillary action into a heparinized, calibrated microhematocrit tube, and a measured volume (2–4 µl) was transferred to heparinized Eppendorf tubes containing 2 µl of a heparin/aprotinin (4 mg/ml and 0.9 mg/ml, respectively) solution made in phosphate buffered saline. Samples were centrifuged at 16,000 × *g* in an Eppendorf microfuge, and serum was collected and frozen at -70°C for later VTG analysis by Western blotting. For this procedure, serum samples, along with a positive control (internal standard) taken from pooled serum of about 20 sexually mature female medaka, were diluted 50 times with SDS-denaturation buffer, loaded onto 7.5% SDS-polyacrylamide gels, and electrophoresed. The gels were blotted electrophoretically onto nitrocellulose filters, and VTG protein bands were detected using mouse monoclonal antibodies made against striped bass VTG (*30*). Bands were visualized using goat anti-mouse alkaline phosphatase IgG antibodies (Bio-Rad Immun-Blot kit; Bio-Rad, Hercules, CA). Quantitation of VTG bands was done using a KODAK Digital Science BandScanner 1 D System (Eastman Kodak, Rochester, NY). Band intensities of male VTG were expressed relative to the intensity of the internal standard.

Reproductive study. Once fish sampling was completed, injection of OP into aquaria was terminated. To eliminate the test chemical, aquaria were thoroughly brushed and siphoned down, followed by flushing with 100 liters well water over 24 hr, which resulted in >99% replacement (*31*). Flow-through conditions (100 l/aquarium/day) were maintained throughout the reproductive studies. Thirty unexposed female medaka were indiscriminately selected for addition to each treatment aquarium for mating with 17 OP-exposed males. Eggs were collected each morning from spawning substrates (6-in cylindrical sponges) placed in each aquarium for 9 consecutive days beginning 2 days after cessation of OP exposure. Eggs were counted and evaluated microscopically to determine percent fertilization as judged by the presence of a perivitelline space located between the chorion and plasma membrane. Aquaria temperatures during the mating period were kept at 27 ± 1°C. Fish were fed twice daily with dry flakes and brine shrimp.

Four groups of 25 viable eggs were collected from each aquarium (200 eggs/treatment), and the chorionic filaments were removed to prevent clumping during the incubation period. Embryos were then transferred to 250-ml hatching jars containing embryo rearing solution (0.1% NaCl, 0.003% KCl, 0.004% $CaCl_2$, 0.163% $MgSO_4$ in distilled water) and incubated with aeration at 24 ± 1°C. Embryos were assessed daily for abnormal development, survival, and hatch. The newly hatched fry were transferred to 1.5-liter chambers to monitor survival, behavior, and growth for a period of 7 days post hatch. Fry were fed a regimen of paramecia, microworms, and brine shrimp. Photographic documentation was performed on abnormal embryo and fry.

Histological analysis. Following final egg collections, 10 male medaka from each exposure concentration (5 from each replicate) were anesthetized with MS-222 and bled for VTG serum analysis. Tails were

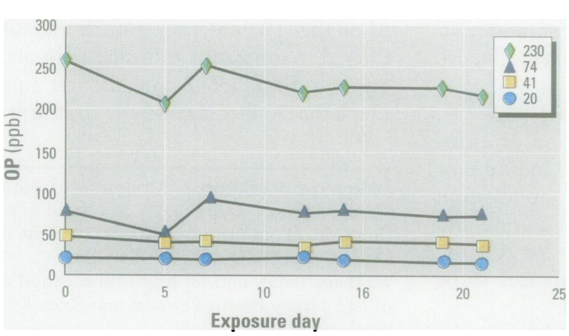

Figure 1. Concentrations of octylphenol (OP) in the exposure aquaria. Concentrations were measured two times per week and were constant throughout the 21-day exposure period. The means and coefficients of variation (CV, standard deviation as a percentage of the mean) for the OP-exposed aquaria are as follows: 230 ppb OP, 229.5 (8.5); 74 ppb OP, 73.9 (17.0); 41 ppb OP, 40.7 (10.0); 20 ppb OP, 20.0 (12.6).

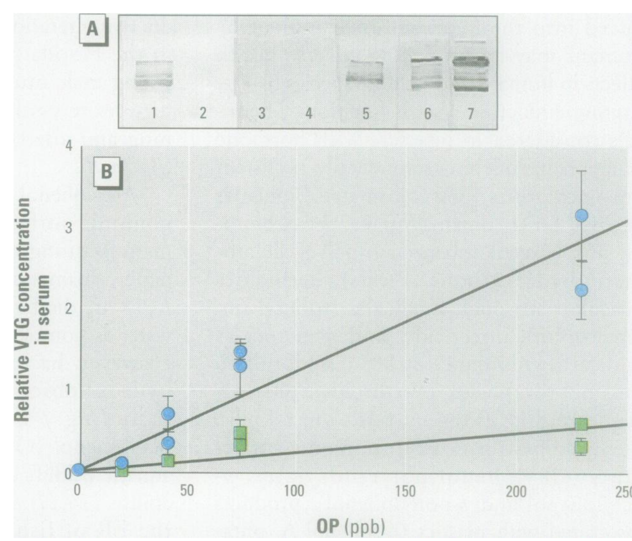

Figure 2. Relationship between octylphenol (OP) concentrations in exposure aquaria and vitellogenin (VTG) levels in serum of OP-exposed male fish. Blood was collected from fish (10/treatment, 5/replicate) immediately after the 21-day exposure period and at the end of the reproductive phase of the study (13 days after cessation of OP exposure), and VTG was measured by Western blot analysis. (A) Composite Western blot of VTG in pooled serum collected from 20 female fish (internal standard) and from individual male fish exposed to increasing concentrations of OP, measured after 21 days of exposure. Lane 1, internal standard; Lane 2, male control; Lanes 3–7, males exposed to OP: Lanes 3 and 4, 20 ppb; Lane 5, 41 ppb; Lane 6, 74 ppb; Lane 7, 230 ppb. VTG bands were quantitated by densitometry (see "Materials and Methods"). (B) Band intensities of male VTG were expressed relative to the intensity of the internal standard. Each data point represents the mean of the VTG serum concentration of five fish; error bars represent standard error. Correlation between OP and VTG is highly significant ($p<0.001$).

removed beyond the caudal peduncle, the body cavity was opened via a midventral slit, and fish were placed into individual cassettes for fixation in 10% neutral buffered formalin. Following standard histological procedures, fish were embedded in paraffin blocks, sectioned at 4 µm, and placed on slides to be stained by hand with Harris Hematoxylin and eosin Y. Coverslips were placed on slides, and the livers and testes of all fish were examined under 40× magnification on a light microscope.

Statistical analysis. Correlations between OP exposure concentrations and serum VTG levels were evaluated by linear regression analysis, and *p*-values were derived from the regression lines. Dichotomous data, including percent fertilization, embryo and fry survival, and incidence of abnormal offspring, were analyzed using logistic regression to determine differences between treatment and control groups (*32*) using SYSTAT 7 for Windows (SPSS, Inc., Chicago, IL).

Results

Average measured concentrations of OP in the flow-through aquaria throughout the 21-day exposure were 20, 41, 74, and 230 ppb (Fig. 1). Western blot detection of VTG in serum of exposed male fish revealed that the estrogen-inducible protein was present in all treatment groups in steadily increasing concentrations (Fig. 2). After OP-exposed male fish were mated with unexposed females, male fish serum was again analyzed for VTG by Western blotting. VTG levels in the male serum had decreased significantly (70–90%) after the 13 days following cessation of OP exposure, with less intense protein bands detected in all treatment groups (Fig. 2). Serum VTG levels before and after the reproductive studies were positively correlated to OP exposure concentrations ($p<0.001$).

During the reproductive phase of the study, control males and females produced approximately two times as many eggs as OP-exposed males and unexposed females over the 9-day egg collection period (Table 1). Individual treatment groups and the controls were significantly different at the $p = 0.07$ level (Student's *t*-test), whereas the four treatment groups combined differed from the control at $p = 0.008$.

Using logistic regression analysis, the increasing OP concentrations were found to be significantly correlated to the decrease in the percent of eggs fertilized by exposed males (Table 1; $p<0.001$). Embryo survival rates decreased as the OP treatments increased (Table 2; $p<0.001$). Increasing occurrence of developmental anomalies, such as bilateral micropthalmia, unilateral anopthalmia, small yolks, and loss of vascular integrity, were observed in embryos of all treatment groups (Table 2; $p = 0.02$). Fry survival did not differ significantly between the control and treatment breeding groups. VTG serum levels measured at the beginning (data not shown) and end of the reproductive phase were significantly correlated to fertilization(Fig. 3, $p<0.001$) and offspring survival (Fig. 4, $p<0.001$). The incidence of abnormal development of offspring was statistically related to VTG serum levels in the male fish ($p = 0.002$; data not shown).

Histopathological analysis of the testes of OP-exposed and control male medaka demonstrated an increase in primary and secondary spermatogonia in fish exposed to concentrations of OP greater than 41 ppb, compared to control fish, indicating inhibition of spermatogenesis. Additionally, oocytes in the testes and germ cell hyperplasia throughout the testes were present in two fish, one in the 74 ppb group and one in the 230 ppb group (Fig. 5).

Discussion

Although designed to clean waste water, sewage treatment plants release large quantities of estrogenic chemicals into the aquatic environment in the form of alkylphenols, by-products of the microbial degradation of APEs. APEs are widely employed as industrial nonionic surfactants used in detergents, paints, herbicides, pesticides, shampoos, and cosmetics. The four largest industrial uses of APEs are in plastics and elastomers, textiles (cleaning, spinning, weaving, finishing), agricultural chemicals (wetters and emulsifiers), and paper (pulping). Additionally, APEs are used extensively in household laundry detergents and hard-surface cleaners (*9*). Over 300 million kg of APEs are produced annually (*33*). Approximately 60% of the APEs released from sewage treatment facilities into the aquatic environment are short-chain APEs such as nonylphenol and octylphenol. Nonylphenol has been identified as an environmental contaminant, with concentrations of 0.11–0.64 µg/l measured in 30 rivers in the United States (*33*), 0.8–15.1 µg/l in final effluents from sewage treatment plants in Texas and Toronto, Canada (*34,35*), and final effluent concentrations of up to 180 µg/liter in the United Kingdom (*36*). Drinking water in the United States has been reported to contain almost 1 µg/l of alkylphenolic compounds (*37*).

Significantly elevated levels of VTG and reduced levels of testosterone have been identified in caged male rainbow trout kept in effluent from sewage works in the United Kingdom, and in male carp captured near a metropolitan sewage treatment plant in the United States (*17,25,38*). The data from these studies suggest that rivers in Great Britain and the United States receive biologically active estrogenic chemicals. The ecological implications of exposure to environmental estrogens have not been adequately investigated (*24*). Two major questions need to be answered: 1) Are there reproductive consequences to aquatic organisms exposed to estrogenic chemicals in the environment? and 2) Can observations on individual organisms predict the effects on population size? This study was designed to help answer the first question by examining whether OP-induced VTG in male fish can be used as a predictive indicator of reproductive impairment. The measured end points in this study (fecundity, fertilization success, and embryo and fry survival) are parameters needed for incorporation into mathematical models used to predict the effects of environmental estrogens on population size and recruitment (*39*).

Table 1. Egg production and fertilization measured over 9 days post octylphenol (OP) exposure

Treatment	Eggs/day[a]	Total eggs	Eggs fertilized	Percent fertilized[b]
Control	61.6 ± 13.4[c]	1108	1065	96.1
OP (ppb)				
20	33.3 ± 8.4[d]	582	526	90.4
41	38.8 ± 8.0[d]	699	630	90.1
74	32.2 ± 8.1[d]	580	497	85.7
230	33.7 ± 6.8[d]	607	504	83.0

[a]Difference between replicates within treatment was not significant; pooled data are shown.
[b]Logistic regression analysis shows a highly significant correlation between increasing OP concentrations and decreases in percent fertilization ($p<0.001$).
[c]Mean ± standard error.
[d]Treatment groups combined are significantly different from controls ($p = 0.008$).

Table 2. Percent survival and incidence of abnormal development of 200 (4 × 25/replicate) viable embryos produced by control and octylphenol (OP)-exposed males and unexposed females

Treatment	Percent survival[a]	No. abnormal embryos[b]
Control	90.5	0
OP (ppb)		
20	73.5	3
41	75.1	5
74	74.4	8
230	63	7

[a]Logistic regression analysis shows a highly significant correlation between increasing OP concentrations and decreases in percent survival ($p<0.001$).
[b]All treatment groups are significantly different.

Male rats exposed *in utero* to the endocrine disruptor 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which may alter reproductive hormone levels through a cytochrome P450-mediated mechanism, exhibited altered sexual behavior (*40*). Endocrine disruption may thus compromise reproductive success by affecting behavior. In the study presented here, the total number of eggs produced by female fish mated with control male fish was approximately twice that produced by females mated with OP-exposed male fish, suggesting that sexual/courtship behavior of the exposed male fish may have been affected by OP concentrations ≥20 ppb. During fetal development in vertebrates, testosterone secreted from the fetal testes causes the sexual (male) imprinting of the brain, which requires the conversion of the male hormone to estradiol by a neuronal P450 aromatase (*41,42*). In teleost fish, many ambisexual species are known. Because of this plasticity in sex differentiation, male imprinting in the teleost brain, which is rich in estrogen receptors (*43*), may require continuous neuronal testosterone conversion to estradiol (*41*). OP-induced decrease of testosterone levels (*44*) in male fish may thus result in feminization of the brain and altered mating behavior. Further studies are needed on the effects of environmental estrogens on mating behavior and on the mechanisms producing the behavioral changes.

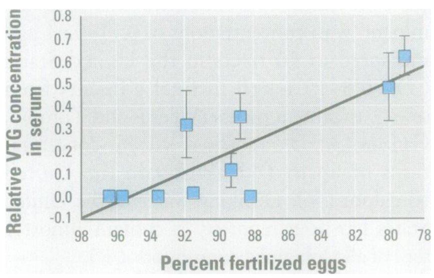

Figure 3. The relationship between vitellogenin (VTG) levels in the serum of male fish (measured at the end of the reproductive phase) and their ability to fertilize eggs (see also Table 1). Male fish (50/treatment, 25/replicate) were exposed to octylphenol (OP; 0, 20, 41, 74, and 230 ppb) for 21 days. Following OP exposure, 5 fish from each replicate (10/treatment) were bled for serum VTG determination (Fig. 2), and 17 males from each replicate were mated with 30 unexposed females. At the end of the reproductive study (13 days after cessation of OP exposure), blood was collected from male fish (10/treatment, 5/replicate) and analyzed for VTG concentration. VTG is the mean concentration in serum of 5 fish; error bars represent standard error. Error bars on five of the data points are too small to be visable. Logistic regression analysis shows a highly significant correlation (p<0.001) between VTG and percent fertilized eggs. The same correlation is found for VTG measured immediately after OP exposure and percent fertilized eggs.

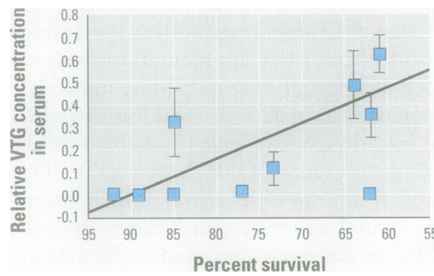

Figure 4. The relationship between vitellogenin (VTG) in serum of male fish (measured at end of the reproductive phase of study) and percent survival. Male fish exposed to octylphenol (OP) for 21 days were mated with unexposed females (17 males and 30 females. At the end of the reproductive study (13 days after cessation of OP exposure), blood was collected from male fish (10/treatment, 5/replicate) and analyzed for VTG concentration. Two hundred viable eggs (4 × 25/replicate) were collected from each of the 10 treatment groups, incubated in embryo rearing solution, and assessed for survival and number of abnormal embryos (see also Table 2). VTG is the mean concentration in serum of 5 fish; error bars represent standard error. Error bars on five of the data points are too small to be visable. Logistic regression analysis shows a highly significant correlation (p<0.001) between VTG and percent survival. A similar correlation is found for VTG measured immediately after OP exposure and survival.

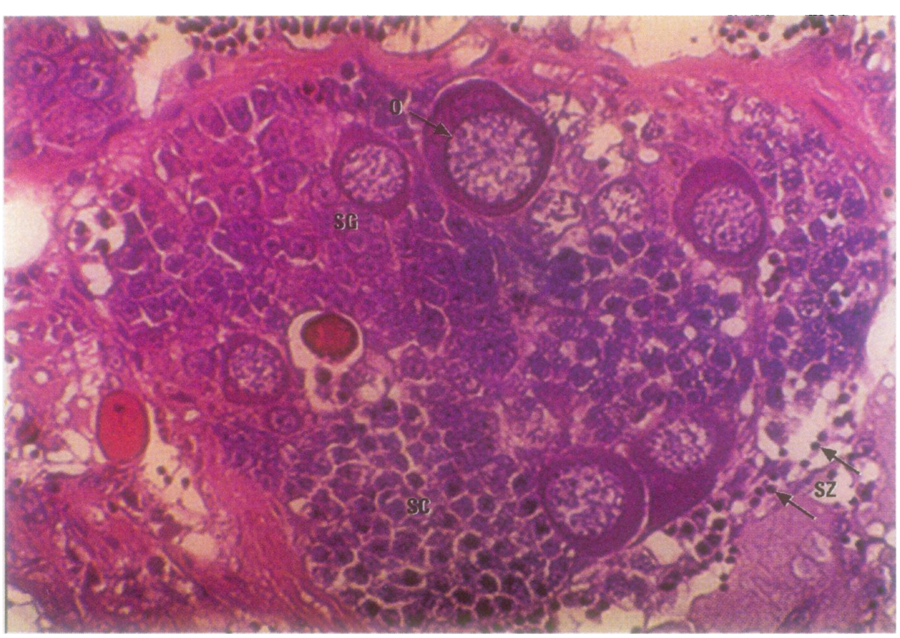

Figure 5. Histopathological analysis of testes from a fish exposed to 74 ppb octylphenol. Abbreviations: O, oocyte; SG, spermatogonia; SC, spermatocyte; SZ, spermatozoa.

VTG production is virtually nonexistent in mature male fish and immature females, but mature oviparous female vertebrates can produce tens of milligrams per milliliter of VTG (*17*). Following the 21-day exposure of male fish to OP, Western blot analysis of serum samples from all exposed male fish revealed increasingly intense VTG protein bands as OP concentrations increased. No VTG was observed in either dilution water or solvent controls. The molecular weight of the two major VTG bands (*M*r ~200,000 and 130,000) corresponds to previously reported values for medaka VTG (*23*). VTG bands of much lesser intensity were observed between the two major bands. The intensities of all VTG positive bands were summed for quantitation. Proteolytical breakdown products of VTG were not observed. Because pure medaka VTG protein was unavailable, vitellogenin in serum collected from sexually mature female medaka was used as the positive control. All VTG band intensities in exposed males were expressed relative to this standard. Doubling of the amount of serum applied to the gel resulted in doubling of the band intensities, indicating that measured VTG concentrations were in the linear range of the standard curve.

On day 21 of the exposure, injection of OP into each of the treatments was discontinued and the compound was flushed from the tanks. Thirteen days later, when mating studies with the exposed male fish were completed, fish were bled again for analysis of VTG serum levels. Although VTG had decreased substantially as compared to the fish sampled just after the exposure period, the correlation between serum VTG and OP exposure concentrations, percentage fertilized eggs, and percent embryo survival was still highly significant.

OP exposure of male fish in the study presented here resulted in 1) a decrease in number of eggs produced by female partners of OP-exposed males, 2) a decrease in percent fertilization, and 3) a decrease in survival of embryos. The effects on fertilization were small, but statistically significant (~15% decrease in highest treatment). The effects of OP exposure on embryo survival (~35% decrease at the highest OP concentration) and number of eggs produced (~50% decrease at all OP concentrations) were more pronounced. Taken together, these data indicate the potential for significant reduction in number of viable offspring, which may result in reproductive output that falls below the critical level required to maintain a viable population. Fish exposed to environmental estrogens chronically or during gonadal development may be more substantially impacted than demonstrated in the 21-day exposure presented here. In addition, OP has been shown to interfere with reproductive function of female fish and rats *(45,46)*, suggesting more dramatic effects to the reproductive capacity of fish when both male and female are exposed to OP. The validity of these hypotheses is under investigation in our laboratory.

Histological analysis of the testes of OP-exposed fish revealed that primary and secondary spermatogonia were more prevalent in the higher treatment fish, indicating inhibition of production of spermatocytes and spermatozoa by OP exposure. Similar observations have been made in rats exposed to OP (*47*). Rainbow trout exposed to OP had a reduction in testicular growth (*16*), and medaka exposed to 50–100 µg/l of nonylphenol (from hatch to 3 months of age) exhibited an 86% incidence of testis–ova (an intersex condition where both testicular and ovarian tissue are present in the gonad) (*48*). The present study demonstrated induction of testis–ova in at least one fully developed adult fish in both the 74 and 230 ppb OP treatment groups.

Approximately 2.5% (20/800) abnormally developing embryos were observed in the three highest OP treatment groups, which is significantly higher than in the controls (0/200) and in laboratory cultures, where approximately 0.1% of naturally spawned embryos show abnormal development (unpublished results). Because OP was absent during the reproductive phase of this study, OP, or more likely a hydroxylated metabolite, may be responsible for damage to sperm DNA. OP may thus be genotoxic to fish. This genotoxic and mutagenic property of OP is further supported by studies which show that Triton X-100, a mixture of OP polyethoxylates, can be hydroxylated by cytochrome P450 enzymes (*49*) and that nonylphenol can increase the activity of cytochrome P450s in fish (*50*). Hydroxylated OP can undergo metabolic redox cycling, generating free radicals such as superoxide and the chemically reactive OP semiquinone/quinone intermediates, which may damage DNA or other macromolecules similar to the 4-hydroxylated metabolite of estradiol (*51,52*).

Conclusion

This study demonstrates a correlation between VTG levels in male fish and impaired reproduction in response to an environmental estrogen. The observed OP-induced decrease in egg production, reduced fertility, and reduced embryo survival may have serious ecological implications. The effects of this estrogenic chemical may be partially reversible in adult fish, as suggested by VTG disappearance following exposure. However, reproductive damage to fish exposed to endocrine-disrupting chemicals during critical stages of gonadal development may be irreversible. Currently, studies are under way in our laboratory to determine the "window of vulnerability" of newly hatched fish and to quantify the effects of early life stage exposure on reproduction following sexual maturity.

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